REMARKS/ARGUMENTS

Prior to the present amendments, claims 13-30, 67-72, and 76 were pending in this application. Claims 13-16, 21, 68, and 76 have been canceled, and claims 27, 67, 69-71 have been amended. All amendments and cancellations were made without prejudice and without acquiescence to any of the rejections, or the reasoning underlying the rejections. Applicants specifically reserve the right to pursue any deleted subject matter in one or more continuing applications.

The claim amendments are fully supported by the specification as originally filed, and do not add new matter. Thus, the recitation of human animals generating antibody diversity primarily by gene conversion is supported by the original claims and throughout the specification, such as, for example, at page 5, lines 18-22; and page 11, lines 14-19. The recitation of "more than one V region gene segment" (claim 27), "more than one V region gene" (claim 67), and "multiple variable (V) gene segments" (claim 67) is supported at least in the paragraph bridging pages 11 and 12, and page 20, lines 19-29 of the specification. The other amendments are of formal nature or concern recitations originally present in other claims.

Request for Withdrawal of the Finality of Rejection

In the Office Action mailed on November 30, 2004 in this case, the Examiner cited a single reference, Singh et al., as allegedly anticipating the claims pending under 35 U.S.C. 102(e). Applicants addressed the rejection, by introducing claims 67-76, explaining the differences between Singh et al. and the invention claimed in the newly added claims, and amending the original claims to exclude birds.

In the Office Action mailed on March 25, 2005, as a result of the claim amendments and Applicants' arguments, the 102(e) rejection was withdrawn, but the Examiner cited three new references. In particular, and the claims were rejected as allegedly being anticipated over two new references (Lonberg et al., or Rader et al.), and/or as allegedly being obvious over Fell et al. in view of Rader et al. According to the Office Action, the new rejections (including the citation of new references) "have been necessitated by applicant's amendments to the claims."

Applicants respectfully disagree.

The current references were equally applicable (or not applicable, as Applicants will argue) to the previously pending claims, since the claims originally encompassed but were not limited to birds. Accordingly, the exclusion of birds from claims from claims 13 and 27, and the claims dependent thereon, did not create a situation when previously not applicable references would have read of the claims as a result of Applicants' claim amendments. Since the newly cited references could have been cited in the previous Office Action, Applicants submit that it is improper to make the present rejections "final." If the finality of the present Office Action is maintained, the prosecution of the present application might suffer unnecessary delays, as a result of the potential non-entry of amendments offered after final rejection.

Accordingly, for the reasons set forth above, Applicants respectfully request the withdrawal of the finality of the present Office Action.

Claim Rejections - 35 U.S.C. § 102

(1) Claims 13-26 and 67-71 have been rejected under 35 USC 102(b) as allegedly being anticipated by U.S. Patent No. 5,569,825 (1996), hereinafter referred to as "Lonberg et al."

Claims 13-16, 21, and 67 have been cancelled. The rejection of the remaining claims is respectfully traversed.

All claims now recite the that the non-human Ig sequences are derived from a non-human animal which generates antibody diversity primarily by gene conversion and/or hypermutation, and that the claimed vectors and methods allow the production of a functional repertoire of humanized antibodies with V region amino acid sequences encoded by more than one V region gene segment.

Since the fact that the non-human animal recited in the claims generates antibody diversity primarily by gene conversion has a profound effect on the structure of the claimed transgenic vectors, steps of the claimed methods, and the structure of the humanized antibodies produced by using the claimed vectors, it is appropriate to start with a brief summary of the differences between antibody diversity generated by gene conversion versus gene rearrangement and hypermutation.

All animals produce antigen-specific antibodies by a two-phase process. The first step involves generation of a primary antibody repertoire in B lymphocytes (B cells). The primary repertoire includes a wide-range of antigen specificities but is not broad enough to contain high-affinity binders for all antigens. Therefore, a second level of diversity is achieved through an antigen-driven selection strategy resulting in preferential selection of B cells that produce high-affinity antibodies. This new population of B cells producing high-affinity, antigen specific antibodies is referred to as a secondary B-cell repertoire, and the antibodies produced as secondary antibody repertoire.

Generally, all vertebrates start the creation of the primary antibody repertoire by recombining V, D, and J gene segments. In mice and humans rearrangement of V, D, and J elements occurs throughout lifetime, while in rabbit, chicken, pig, sheep, cow, and certain other vertebrates, gene rearrangement stops around the time of birth. In mice and humans, gene rearrangement results in considerable diversity as hundreds of VDJ genes are randomly recombined and genes are imprecisely joined together. However, in certain other vertebrates, such as rabbit, chicken, pig, sheep, and cow, this first step of VDJ recombination does not lead to significant diversity because only a limited number of genes are employed. In order to enhance diversity of the primary repertoire, animals of this latter group use a second step to modify antigen-binding regions through templated (gene conversion) and non-templated (hypermutation) mutational processes. Such animals are referred to in the present application as "generating antibody diversity primarily by gene conversion," or, briefly, as "gene converting" animals. Gene conversion creates broad diversity by modifying all three antigen-binding sites of the VDJ region.

The process of gene conversion transfers sequence information encoded (i.e. templated nucleotide substitutions) in upstream V genes to the rearranged exons. A rearranged V gene undergoes about 10 gene-conversion events during B cell development, resulting in changes to each of the antigen-binding sites or complementarity-determining regions (CDRs). Insertion of sequences starts at sites in the recipient V gene where it shares extensive sequence homology with the donor V element and stops where sequence homology falls below a minimum threshold. For this reason, gene conversion selectively modifies CDR regions while leaving framework regions unaltered. The diversification of the primary antibody repertoire in gene converting

animals is significantly greater than the diversification of primary antibodies through rearrangement in rodents and humans because it allows the combination of fragments of several V gene segments. As a consequence of gene conversion most high-affinity antibodies in gene converting animals contain a variable region which is primarily identical to a polypeptide sequence encoded by fragments of more than one V gene segments. This is significantly different from mice and humans (which do not use gene conversion for antibody diversification) where V regions of antibodies are always encoded by a single V element.

This significant difference is clearly reflected by the language of the claims currently pending.

In particular, the independent claims pending in this application recite the following elements:

Claim 67, on which all transgenic vector claims depend, is directed to transgenic vectors carrying a humanized Ig locus, where the Ig locus is characterized by: (1) being derived from an Ig locus or a portion of an Ig locus of a non-human animal which generates antibody diversity primarily by gene conversion and/or hypermutation; (2) having regulatory sequences identical to regulatory sequences of the Ig locus of a non-human animal which generates antibody diversity primarily by gene conversion and/or hypermutation; (3) comprising two or more Ig gene segments including multiple variable (V) gene segments and multiple J gene segments, integrated into the Ig locus or portion of an Ig locus of the non-human animal, wherein (4) at least one of the gene segments is a human gene segment, (5) the human gene segment(s) is/are flanked and separated by non-coding sequences from the non-human animal, and (6) the Ig gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration. As a result, (7) the humanized Ig locus is capable of undergoing gene conversion and producing a unique repertoire of humanized immunoglobulins with V region amino acid sequences encoded by segments of more than one V region gene.

Method claim 27, on which all method claims depend, is directed to the preparation of a transgenic vector comprising a humanized Ig locus capable of producing a functional repertoire of humanized antibodies in a non-human animal generating antibody diversity primarily by gene conversion. According to this method: (1) a DNA fragment comprising an Ig locus or a portion

of an Ig locus is obtained from a non-human animal which generates antibody diversity primarily by gene conversion and/or hypermutation, wherein the Ig locus obtained (a) comprises at least one V gene segment, at least one J gene segment and at least one constant region gene segment, and (b) regulatory sequences from the non-human animal; and (2) at least one human Ig gene segment is integrated into the DNA fragment of the non-human animal, where the human Ig gene segment includes (a) a human V gene segment, and (b) is flanked by non-coding sequences from the non-human animal; and wherein (3) the elements of the vector are operably linked so as to produce a functional repertoire of humanized antibodies with V region amino acid sequences encoded by more than one V region gene segment, in the non-human animal.

To anticipate a claim, a reference must teach each element of the claim (*Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). Applicants submit that Lonberg et al. does not meet this requirement.

Lonberg et al. concerns non-human transgenic animals in which endogenous immunoglobulin production is suppressed, and which carry essentially fully human immunoglobulin fragments. While all work was done with mice (non-gene converting), the reference also lists bovine, ovine and porcine species, and rabbit (gene converting) as transgenic non-human animals. However, Lonberg et al. did not recognize the significance of the fact that bovine, ovine and porcine species, and rabbit are gene converting animals, and as a consequence, use a completely different mechanism from humans and mice for generating antibody diversity. In particular, Lonberg et al. did not recognize that introduction of human immunoglobulin loci into gene converting animals, as described in the Lonberg et al. specification, is not expected to result in any significant gene diversification by gene conversion. Indeed, human immunoglobulin loci are optimized for the expression of diverse antibody repertoire based on gene rearrangement. There is no evidence in Lonberg et al. or otherwise, of gene conversion occurring during normal antibody diversification in humans and mice.

The vectors of Lonberg et al. <u>do not contain</u> regulatory sequences identical to regulatory sequences of the Ig locus of a non-human animal which generates antibody diversity primarily by gene conversion; <u>do not contain</u> human V gene segments flanked and separated by non-coding sequences from a non-human animal which generates antibody diversity primarily by

gene conversion and/or hypermutation; and, as a result of these structural references, are not capable of producing of a functional repertoire of humanized antibodies in a non-human animal which generates antibody diversity primarily by gene conversion.

In the vectors claimed in the present application, non-coding sequences, including regulatory sequences, of the recipient non-human animal, which generates antibody diversity primarily by gene conversion, are retained, and only coding sequences are replaced by coding sequences of a human Ig polypeptide. Accordingly, when using these vectors, only coding sequences from the endogenous Ig locus of the recipient animal are replaced by human gene sequences. These structural differences are very significant, because Applicants' immunoglobulin loci allow generation of a diversified human or humanized antibody repertoire by gene conversion in gene converting animals. In contrast, the vectors of Lonberg et al. do not result in immunoglobulin loci that allow generation of a diversified antibody repertoire by gene conversion in gene converting animals. Rather, the vectors of Lonberg et al., which contain a complete human Ig locus, without non-coding sequences of the non-human animal surrounding the human sequences, are suitable only for diversification of the antibody repertoire by gene rearrangement.

Since the vectors described in Lonberg et al. are not suitable for generating humanized antibodies in animals generating antibody diversity primarily by gene conversion, and, due to the discussed structural differences, do not anticipate the claims pending in this application, the Examiner is respectfully requested to reconsider and withdraw the present rejection.

(2) Claim 76 was rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Rader et al. (May 2000) J. Biol. Chem., Vol. 275, No. 18, 13668-13676.

The cancellation of claim 76, which was done without prejudice and without acquiescence to the rejection, moots this rejection.

Claim Rejections - 35 U.S.C. § 103

Claims 27-30 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 5,202,238 (1993) (Fell et al.), in view of Radar et al. (May 2000) J. Biol. Chem., Vol. 275, No. 18, 13668-13676.

Fell et al. was cited for its teaching of making transgenic vectors by providing a phage containing the entire mature gene encoding a murine antibody heavy chain, and replacing the constant region of the murine heavy chain by the human constant region by homologous recombination. Rader et al. was cited as allegedly providing motivation for making chimeric rabbit antibodies over murine antibodies, and for its teaching that the rabbit Ig gene repertoire is well characterized.

As discussed above, transgenic vectors designed to produce humanized antibodies in non-human animals which generate antibody diversity primarily by gene conversion must be structurally significantly different from vectors used in mice, which generate antibody diversity by gene rearrangement and hypermutation. Neither Fell et al. nor Rader et al. has any teaching indicating the recognition of this significant difference. Accordingly, even if it is assumed that Rader et al. provides motivation for producing humanized antibodies in rabbits, the combination of Fell et al. and Rader et al. would be inoperable, since the vectors of Fell et al. would not produce a functional repertoire of humanized antibodies in rabbits or other animals which generate antibody diversity primarily by gene conversion.

Since the combination of Rader e al. and Fell et al. does not enable the production of humanized antibodies in non-human animals which generate antibody diversity primarily by gene conversion, the reconsideration and withdrawal of the present rejection is respectfully requested.

All claims pending in this application are prima facie condition for allowance, and an early action to that effect is respectfully solicited.

Although no fees are believed to be due at this time, please charge any fees, including any fees for extension of time, or credit overpayment to Deposit Account No. **08-1641**, referencing **Attorney's Docket No. 39691-0005 A**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: June 23, 2005

By: _____
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